Regression of Dietary Copper Restriction-Induced Cardiomyopathy by Copper Repletion in Mice[1]

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ABSTRACT Dietary copper deficiency (CuD)[3] leads to cardiac hypertrophy in various animal models. We showed recently that heart failure develops after hypertrophy in FVB mice fed a CuD diet. The present study was undertaken to determine whether CuD-induced cardiac failure is reversible upon copper repletion (CuR). Dams of FVB mice were fed a CuD diet (0.3 mg/kg) starting from d 3 postdelivery; the weanling pups were fed the same diet until CuR with 6.0 mg/kg Cu in the diet at 4 or 5 wk of age. CuR at 4 wk of age prevented the body weight loss; at 5 wk of age, it resulted in the regaining of the lost weight caused by CuD. A significant regression of CuD-induced cardiac hypertrophy was observed in the CuR mice. Histopathological examination revealed that CuR eliminated CuD-caused lipid deposition in the myocardium, and electron microscopy demonstrated that CuD-induced ultrastructural changes such as mitochondrial swelling and organelle structural disarray were all reversed in the CuR mice. Hemodynamic analysis showed that the CuD-depressed systolic and diastolic parameters such as the maximal rate of left ventricular pressure rise (+dP/dt) and decline (−dP/dt), and the contraction and relaxation times were completely recovered in the CuR mice. Furthermore, the CuD-blunted myocardial responses to the β-adrenergic agonist, isoproterenol, were also restored in the CuR mice. This study thus demonstrates for the first time that CuR results in the regression of heart failure induced by CuD as demonstrated by the reversal of depressed cardiac hemodynamic and contractile function and the restored responsiveness to β-adrenergic stimulation. J. Nutr. 134: 855–860, 2004.

KEY WORDS: • *cardiomyopathy* • *cardiac function* • *copper deficiency* • *copper repletion* • *heart hypertrophy*

Dietary copper deficiency (CuD)[3] in animal models leads to concentric cardiac hypertrophy along with characteristic alterations in myocardial morphology and biochemical and signaling pathways (1–7). Multiple processes are involved in CuD-induced pathologic changes; however, the major causes leading to these processes have not been identified. These components include connective tissue metabolism, antioxidant activity, and energy metabolism. In each of these systems, a cuproenzyme's function is altered: the extracellular matrix collagen-elastin crosslinking enzyme, lysyl oxidase (8,9), which is involved in connective tissue metabolism; the cytosolic antioxidant enzyme copper/zinc superoxide dismutase; and the mitochondrial respiratory chain enzyme cytochrome c oxidase.

The contribution of each of these copper restriction–affected components to the overall CuD-induced cardiac hypertrophy and the triggers that lead to each of these pathologic changes are the current focus of CuD-induced cardiomyopathy research. Although the mechanistic insights into CuD-induced cardiac hypertrophy are critical for understanding the pathogenesis, studying the progression of CuD-induced cardiac hypertrophy is another important aspect. In addition, it is important to know whether CuD-induced cardiomyopathy is reversible.

In pressure overload–induced heart hypertrophy, there are several stages in the development of the hypertrophic cardiomyopathy. These include the compensatory hypertrophic stage and the decompensated heart failure stage. The nature of the pressure overload–induced heart hypertrophy is concentric. It is interesting to note that CuD-induced heart hypertrophy is also concentric, although the latter is not accompanied by hypertension.

In previous studies, we attempted to determine whether CuD-induced heart hypertrophy would progress to decompensated heart failure, as observed in pressure overload–induced hypertrophic cardiomyopathy. To that end, we used a mouse model and performed functional analysis in mice with heart hypertrophy induced by dietary copper restriction. The results showed that a significant decrease in the left ventricle systolic pressure and contractility occurred in the hypertrophic heart as the pathologic changes progressed. Furthermore, these se-

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[3] Abbreviations used: β-AR, β-adrenergic receptor; CuD, copper deficient (deficiency); CuA, copper adequate; CuR, copper repleted (repletion); EDP, end diastolic pressure.

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verely hypertrophic copper-deficient hearts had a blunted response to the stimulation of the β-adrenergic agonist, isoproterenol. These results thus demonstrated that CuD-induced heart hypertrophy, like pressure overload–induced heart hypertrophy, further develops to decompensated heart failure (6).

Although defining the stages of CuD-induced cardiomyopathy is an important aspect for understanding the progression of this pathogenesis, a relevant and more practical question is whether CuD-induced cardiomyopathy is reversible. Addressing this question will provide further insights into the role of copper in the regulation of myocardial function and pathogenesis. The reversibility of certain aspects of CuD-induced cardiac changes by copper repletion (CuR) was investigated previously (10,11). Cardiac ultrastructural abnormalities such as structural damage to mitochondria and myofibrils and cardiac electrophysiologic changes were partially reversed upon CuR (10). The present study was undertaken in an attempt to determine whether CuD-induced changes in hemodynamics and contractility are reversible upon reintroduction of adequate copper in the diet in the mouse model. This study is designed to address several important questions: *1*) Can reintroducing copper into the diet of copper-deficient mice attenuate or reverse heart hypertrophy and its related systemic changes? *2*) If heart hypertrophy is attenuated, is the altered heart function under nonstress conditions ameliorated or normalized? *3*) Under stress conditions (β-agonist stimulation) do these mice regain their sensitivity to adrenergic stimulation?

MATERIALS AND METHODS

Animals and treatment. Friend Virus B-type (FVB) mice were bred and maintained at the University of Louisville animal facilities housed in plastic cages at 22°C with a 12-h light:dark cycle. Because this mouse strain is characterized by a high reproductive rate and large litters, it was chosen for use in this study. The AIN-93 diet was prepared by the U.S. Department of Agriculture Human Nutrition Research Center (Grand Forks, ND) according to a previously published report (12). The composition of the diet was modified to contain 0.3 mg Cu/kg diet [copper-deficient (CuD) diet] or 6.0 mg Cu/kg diet [copper-adequate (CuA) diet]. Dams of the pups were fed the CuD diet starting on the d 3 postdelivery. After the pups were weaned on d 21, they were fed the same diet until they were 4 wk old at which point some of the CuD mice continued to be fed the CuD diet and others were switched to the CuA diet. Mice that were switched from the CuD diet to CuA at 4 wk of age continued to consume the CuA diet for either 2 or 4 wk. Analysis of the effect of CuR on heart morphology, function, and mineral concentrations was performed on mice that were copper-repleted starting from wk 4 for an additional 2 wk (for morphology and mineral analysis) or both 2 and 4 wk (for functional analysis). Mice had free access to double-distilled water. Cages, feeding jars, and water bottles were rinsed regularly with water containing EDTA first and then with distilled water. Body weight was monitored weekly starting from wk 3 after birth. All procedures were approved by the AAALAC certified University of Louisville Institutional Animal Care and Use Committee.

Assessment of left ventricle performance. Heart performance measurements were carried out using a surgical procedure, as previously described (6).

Assessment of isoproterenol response. Isoproterenol was delivered through a femoral vein catheter (0.1 μL/g body weight) with a microliter syringe pump (Harvard Aparatus-22). It was administered at a constant rate of infusion in varying concentrations of 0.08, 0.16, and 0.32 ng Iso/(min · g body weight) given for a total of 3 min for each dose. Mice were allowed to recover for 10–15 min before administration of each successive dose. Heart performance under the stimulation of isoproterenol was analyzed by the same surgical procedure described previously (6).

Assessment of morphology changes. Hearts were excised from pentobarbital-anesthetized mice, washed with saline solution, and placed in 10% formalin. Hearts were cut transversely close to the apex for visualization of the left and right ventricles. Several sections of heart (4- to 5-μm thick) were prepared and stained with hematoxylin and eosin for visualization using light microscope. For examination by electron microscopy, a tissue sample preparation procedure described previously was used (13).

Cu and other mineral concentrations in the heart and in the serum. Minerals including Cu concentrations were determined in the heart using inductively coupled argon plasma emission spectroscopy (model 35608, Thermo ARL-VG Elemental) after lyophilization and digestion of the tissues with nitric acid and hydrogen peroxide (14). Dietary Cu concentrations were analyzed using a dry-ashing procedure, which was followed by dissolution of the residue in aqua regia and measurement by atomic absorption spectrophotometry (model 503; Perkins Elmer).

Statistical analysis. Data are expressed as means ± SEM. Body weight, heart weight, the heart weight/body weight ratio, and cardiac function data were analyzed by 1-way ANOVA using Microsoft Excel 97 followed by a two-tailed Student's *t* test. Differences were considered significant at $P < 0.05$.

RESULTS

Effect of CuR on CuD-induced heart hypertrophy. Dietary copper deficiency caused body weight loss starting from 5 wk of age (**Fig. 1**). By 6 wk, CuD mice were lethargic and died soon after. Reintroduction of the CuA diet to CuD mice at 5 wk of age resulted in a regain of the lost body weight. When the CuA diet was reintroduced at 4 wk of age, the body weight gain of the mice did not differ from that of the control mice fed CuA diet continuously, i.e., the CuA and copper-repleted groups did not differ from 5 to 8 wk of age. At 4 wk of age, CuD mice heart weights and the heart weight to body weight ratio were elevated compared with age-matched CuA controls. After reintroduction of CuA diet to the CuD mice at 4 wk of age, the heart weight and the heart weight to body weight ratio decreased after these mice continued to consume the CuA diet for 2 wk (**Table 1**). Continuation of the CuA feeding to CuD mice for an additional 2 wk normalized the heart weight to body weight ratio; however, the absolute heart weight of the CuR mice remained higher than that of control mice, although the two groups did not differ significantly (Table 1). Mineral analysis showed that CuR lead to an increase in heart Cu concentrations that were decreased con-

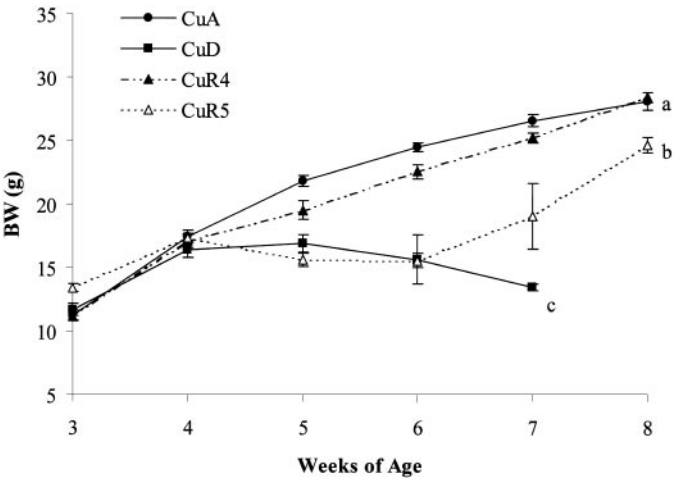

FIGURE 1 Effect of dietary CuR beginning at 4 or 5 wk of age on CuD-induced body weight change in mice. CuR4, repletion starting at wk 4; CuR5, repletion starting at wk 5. Values are means ± SEM, n = 3–10. Means without a common letter differ, $P < 0.05$.

TABLE 1

The effect of copper repletion for 2 (CuR2) or 4 wk (CuR4) on copper deficiency (CuD)-induced changes in absolute and relative heart weights in FVB mice[1]

	4 wk of age		6 wk of age		8 wk of age	
	CuA	CuD	CuA	CuR2	CuA	CuR4
n	5	13	8	14	6	10
Heart, *mg*	99.5 ± 6.7[d]	167.9 ± 7.2[a]	122.3 ± 3.8[c]	141.1 ± 5.1[b]	129.5 ± 5.7[c]	140.0 ± 6.4[bc]
Heart/body, *mg/g*	5.6 ± 0.2[b]	9.2 ± 0.5[a]	5.3 ± 0.1[b]	6.0 ± 0.2[b]	4.7 ± 0.2[c]	5.2 ± 0.2[b]

[1] Values are means ± SD. Means in a row without a common letter differ, $P < 0.05$.

siderably by CuD (**Table 2**). In addition, CuR caused a significant increase in zinc concentrations in the heart. Serum Cu levels were almost nondetectable in the CuD mice whereas in the CuR mice, Cu concentrations returned to the CuA control values.

Effects of CuR on CuD-induced functional alterations. Hemodynamic analysis revealed that the heart rate was significantly decreased in CuD mice (by 21%) after 4 wk of age, and normalized after reintroduction of Cu in the diet for 2 wk. A marked attenuation of maximum and minimum arterial pressure in the CuD mice after 4 wk of age was observed (**Table 3**). Reintroduction of Cu in the diet resulted in the normalization of both parameters. Similarly, left ventricle peak systolic pressure was elevated from 80 mmHg in the CuD mice to 94 mmHg in CuR mice at 2 wk and to 98 mmHg in CuR mice at 4 wk ($P < 0.05$). In addition to hemodynamic parameters, left ventricle contractility as assessed by +dP/dt was depressed by 32% and −dP/dt was 39% in the CuD mice. CuR for 2 wk resulted in a partially normalized contractility, whereas CuR for 4 wk completely normalized the contractility.

In addition to the depressed −dP/dt, which serves as an index of ventricular relaxation, another index, Tau, the parameter of ventricular compliance, was significantly increased in CuD mice and normalized in CuR mice. The duration of relaxation and of half-relaxation were prolonged in CuD mice (by 38 and 40% respectively) and normalized in the CuR mice. End diastolic pressure was significantly increased from 4 ± 1 to 6 ± 3 mmHg in CuD mice ($P < 0.05$) and Cu reintroduction normalized this change to 3 ± 1 mmHg in CuR mice, which was comparable to the controls.

CuR restoration of response to β-adrenergic stimulation. CuD mice had a blunted response to the β-adrenergic agonist, isoproterenol, whereas CuA mice had a dose-dependent response to isoproterenol (**Fig. 2**). CuR significantly restored the CuD-blunted β-adrenergic responses. The responses to isoproterenol stimulation were partially recovered in mice fed CuR for 2 wk, but completely recovered in mice fed CuR for 4 wk; there was no significant difference in these responses between CuR mice allowed to recover for 4 wk and their age-matched CuA controls.

Morphology by light and electron microscopes. We showed previously that copper-deficient hearts display severe lipid droplet accumulation that can be visualized by light microscope; the same change was observed in this study (**Fig. 3**). In the CuR mice, the lipid droplets were significantly reduced. The analysis of ultrastructural changes through electron microscope examination showed that changes in mitochondrial structures induced by CuD were significantly attenuated in the CuR mice (Fig. 3). These CuD-induced mitochondrial damages included mitochondrial swelling, disarray and decreased number or disappearance of cristae. In addition, changes in other organelles were also noted, such as loss of myofibrils and cytoplasmic vacuolization. CuR for 2 wk reversed all of these injuries to ultrastructural components induced by CuD (Fig. 3).

DISCUSSION

The results obtained from this study show for the first time that CuD-induced alterations in hemodynamics and contractility are reversible upon CuR and that CuR leads to improved adrenergic responsiveness of the heart. An important finding from this study is that CuD-induced cardiac hypertrophy was partially reversed and that CuD-induced depression in cardiac function was completely recoverable. The reversal of the cardiac functional parameters was associated with improvement in myocardial histology and ultrastructures that were severely affected by CuD. The improved cardiac morphology and func-

TABLE 2

Changes in Cu, Zn, and Fe concentrations in the serum and heart of copper-deficient (CuD) FVB mice after copper repletion for 2 wk (CuR2)[1]

	Serum			Heart		
	CuA	CuD	CuR2	CuA	CuD	CuR2
	μmol/g protein			*μmol/g dry weight*		
Cu	87.7 ± 44[a]	0.4 ± 8[b]	97.5 ± 50[a]	0.2 ± 0.02[a]	0.08 ± 0.004[b]	0.4 ± 0.08[a]
Zn	310 ± 144	175.6 ± 50	325.8 ± 81	1.1 ± 0.1[a]	1.3 ± 0.07[a]	2.6 ± 1[b]
Fe	1633.3 ± 363	1105.9 ± 333	1299.3 ± 582	5.1 ± 0.7	5.1 ± 0.9	5.7 ± 0.6

[1] Values are means ± SD, n = 3–5. Means in a row without a common letter differ, $P < 0.05$.

TABLE 3

Hemodynamic, contractile, and chronotropic parameters of copper-deficient (CuD) FVB mice after copper repletion for 2 (CuR2) or 4 wk (CuR4)[1]

	4 wk of age		6 wk of age		8 wk of age	
	CuA	CuD	CuA	CuR2	CuA	CuR4
Heart rate, *beats/min*	525.7 ± 17.6[a]	415.0 ± 31.1[b]	477.4 ± 6.9[b]	462.2 ± 13.4[b]	497.9 ± 23.1[a]	462.1 ± 8.5[b]
Arterial P max,[2] *mmHg*	88.1 ± 2.7[a]	67.1 ± 1.7[b]	87.7 ± 1.8[a]	85.3 ± 3.2[a]	89.2 ± 4.9[a]	86.8 ± 1.6[a]
Arterial P min, *mmHg*	61.4 ± 3.7[a]	29.2 ± 2.2[b]	61.6 ± 3.0[a]	58.6 ± 4.0[a]	62.8 ± 6.0[a]	57.5 ± 2.7[a]
VPSP, *mmHg*	99.4 ± 3.8[a]	79.7 ± 3.2[b]	98.5 ± 2.8[a]	93.5 ± 7.0[a]	99.3 ± 3.0[a]	98.5 ± 1.8[a]
VMDP, *mmHg*	−2.1 ± 1.2	0.5 ± 1.1	−1.7 ± 1.4	−0.4 ± 0.5	−3.2 ± 1.0	−2.9 ± 1.0
VEDP, *mmHg*	3.6 ± 0.3[a]	6.4 ± 0.8[b]	3.7 ± 0.7[a]	3.1 ± 0.3[a]	2.8 ± 0.8[a]	3.3 ± 0.3[a]
+dP/dt, *mmHg/s*	6071.9 ± 480.1[a]	4146.3 ± 388.0[b]	6190.4 ± 569.9[a]	5333.6 ± 354.4[a]	6897.5 ± 400.9[a]	6924.5 ± 337.9[a]
−dP/dt, *mmHg/s*	5151.3 ± 392.6[a]	3120.9 ± 298.7[b]	5264.6 ± 396.0[a]	4574.4 ± 254.7[a]	5805.7 ± 286.1[a]	5768.3 ± 314.3[a]
DCON, *ms*	32.7 ± 1.0	51.9 ± 8.2	35.7 ± 2.4	38.9 ± 1.3	32.4 ± 1.9	35.9 ± 1.4
Tau, *ms*	14.5 ± 0.6[a]	22.4 ± 1.1[b]	14.2 ± 0.5[a]	15.1 ± 0.5[a]	13.8 ± 0.7[a]	14.0 ± 1.1[a]
Half-R, *ms*	26.6 ± 1.1[a]	37.6 ± 2.8[b]	29.5 ± 2.5[a]	28.8 ± 1.3[a]	31.3 ± 2.8[a]	33.2 ± 1.1[ab]
DREL, *ms*	49.9 ± 2.6[a]	68.7 ± 5.5[b]	54.2 ± 2.4[a]	56.6 ± 1.8[a]	50.0 ± 3.3[a]	52.3 ± 0.7[a]

[1] Values are means ± SEM, $n = 5–14$. Means in a row without a common letter differ, $P < 0.05$.

[2] Parameter abbreviations: arterial P max, maximum arterial pressure; arterial P min, minimum arterial pressure; VPSP, ventricular peak systolic pressure; VMDP, ventricular minimum diastolic pressure; VEDP, ventricular end diastolic pressure; +dP/dt, maximum dP/dt during ventricular contraction; −dP/dt, maximum negative dP/dt during ventricular relaxation; DCON, duration of contraction; Tau, time constant of relaxation; half-R, duration of half-relaxation; DREL, duration of relaxation.

tion were associated with systemic improvement in CuD mice in which the lost body weight was rapidly and completely regained. If these mice had continued to consume the CuD diet, they would have died within the experimental time period.

Cardiac hypertrophy is one of the most characteristic findings in CuD-induced cardiomyopathy in the rat and mouse model (1,2). It has been traditionally thought that cardiac hypertrophy is an adaptive response that benefits the heart by normalizing wall tension; however, increasing evidence exists that hypertrophy occurs either inadequately or excessively (15–17). Epidemiologic studies showed that ventricular hypertrophy is associated with adverse outcomes in patients and an increased risk of sudden death or heart failure (15,17). In the case of concentric hypertrophy occurring in patients with mitral stenosis, approximately one third have inadequate ejection fraction, reduced output, and a reflex increase in peripheral vasoconstriction that leads to an increase in vascular

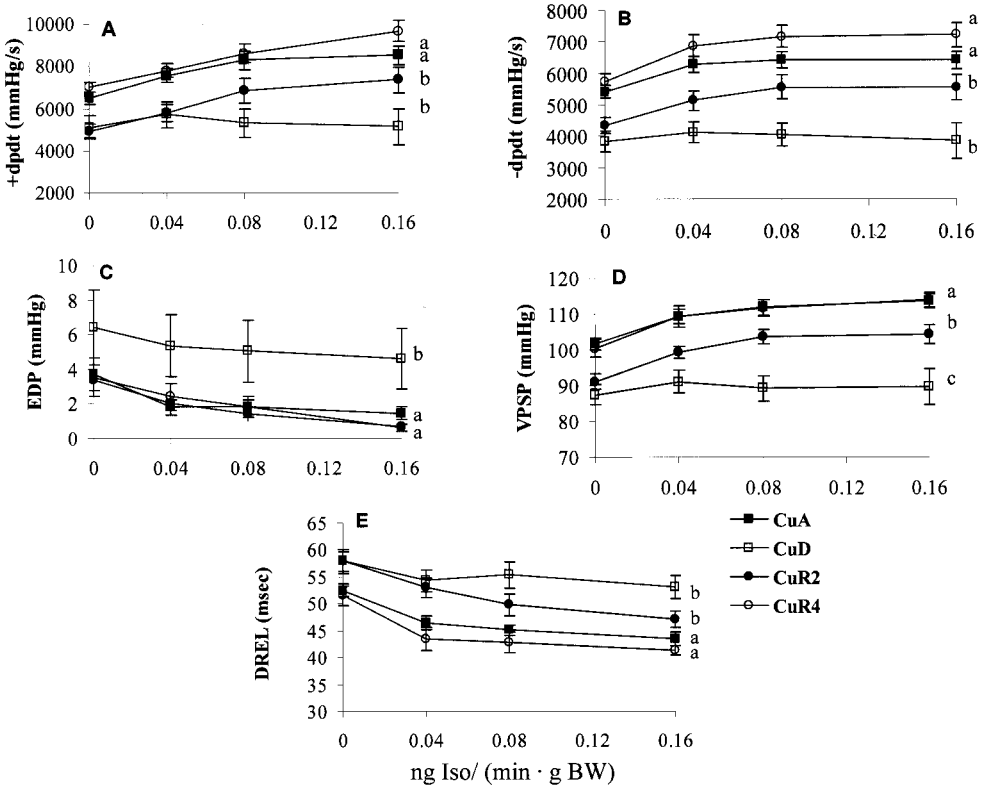

FIGURE 2 Recovery of myocardial response to isoproterenol stimulation in CuD mice receiving dietary CuR beginning at 4 or 5 wk of age. Shown are alterations in the rate of pressure increase (+dP/dt) (*A*) and decrease (−dP/dt) (*B*), alterations in end diastolic (EDP) (*C*) and ventricular peak systolic pressure (VPSP) (*D*), and change in the duration of relaxation (DREL) (E). CuR2, repleted for 2 wk; CuR4, repleted for 4 wk. Values are means ± SEM, $n = 4–10$. Means without a common letter differ, $P < 0.05$.

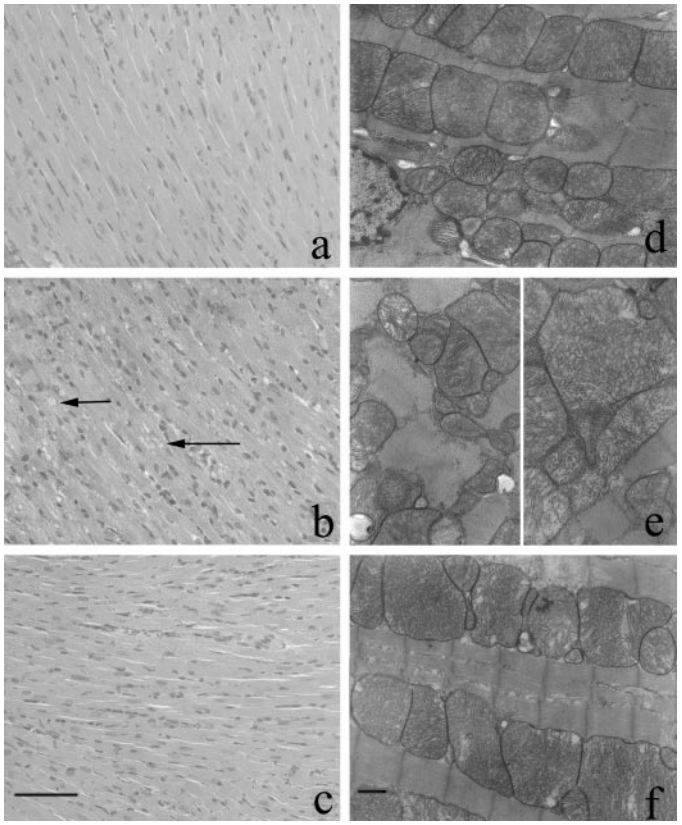

FIGURE 3 Light and electron micrographs from CuA (*a*, *d*), CuD (*b*, *e*) and CuR for 2 wk (*c*, *f*) CuD mice receiving dietary CuR beginning at 4 wk of age. Arrows point to lipid deposition in CuD hearts. Electron micrographs show mitochondrial swelling and structural changes and myofibril damage and disarray as well as vacuolization in CuD mice. Bar in light micrographs = 0.05 mm, in electron micrographs = 1 μm.

resistance to outflow (16). In such cases, it is thought that the hypertrophy that occurs is inadequate. The hypertrophy associated with dietary CuD is of a concentric nature, which is similar to that found in the pressure overloaded heart and in aortic stenosis (1). We showed previously that cardiomyopathy in the dietary CuD model is characterized by severe hypertrophy and is accompanied by deteriorations in cardiac systolic and diastolic functions (6). The deterioration in cardiac function subsequent to the development of cardiac hypertrophy in the CuD mice therefore defined a transition from cardiac hypertrophy to end-stage heart failure.

End-stage cardiac failure is a "pathophysiological state in which an abnormality of cardiac function is responsible for failure of the heart to pump blood at a rate commensurate with the demands of the metabolizing tissues" (18). End-stage heart failure is a decompensatory stage of cardiac hypertrophy characterized by altered hemodynamics, impaired muscle function, and increased fibrosis (19,20). Furthermore, heart failure is associated with the activation of the sympathetic nervous system, resulting in increased levels of circulating catecholamines (norepinephrine) during rest and exercise as well as a concomitant decrease in β-adrenergic responsiveness in the failing heart (21). It was suggested that alterations in β-adrenergic receptor (β-AR) signaling occur in heart failure, including alterations in the expression (degradation or down-regulation of gene expression) and function (through phosphorylation) of the receptor, G protein, or adenylyl cyclase. The desensitization or the deactivation of the β-AR is thought to occur in heart failure because of continuous stimulation by the increased circulating catecholamines.

We showed previously that dietary copper deficiency impaired both systolic and diastolic functions, as assessed by both hemodynamic and contractile indices (6). Furthermore, our assessment of cardiac function of CuD mice under stress conditions, i.e., β-adrenergic stimulation, further corroborated our assertion that CuD is associated with heart failure because CuD mice had a blunted response to stimulation by the β-adrenergic agonist, isoproterenol. These results also indicate alterations in β-AR signaling in the CuD mice.

Surprisingly, all of the aforementioned changes can be prevented, or reversed, after Cu is reintroduced into the diet. Indicators of systolic function such as left ventricular maximum systolic pressure and rate of rise of ventricular pressure (+dP/dt) in the CuR mice were completely recovered and did not differ significantly from the age-matched CuA control group. In addition to the favorable alterations in systolic function, diastolic function was similarly recovered. Diastolic dysfunction is characterized by slowed or incomplete relaxation, abnormal left ventricular filling, and altered passive elastic properties of the heart (22,23). We previously reported a significant increase in end diastolic pressure (EDP), a decrease in the rate of fall of left ventricle pressure (−dP/dt), and elongation of both myocardial relaxation time and time to half-relaxation. Myocardial relaxation depends mainly on the following factors: *1*) inactivation processes within myocytes, i.e., the dissociation of actomyosin bridges and the lowering of Ca concentration by active uptake by the sarcoplasmic reticulum; *2*) loading conditions, mainly afterload; and *3*) restoring forces that depend on the elastic properties of the myocardium, which have been linked to extracellular matrix components such as collagen (22,23). Both EDP and −dP/dt were returned to normal levels by CuR, which would indicate in addition to unloading of the left ventricle as marked by the decrease in EDP, an intrinsic amelioration in ventricular relaxation mechanisms as shown by the normalization of −dP/dt. Furthermore, CuR led to the progressive improvement of the response to β-adrenergic stimulation. The restoration of the β-AR level and/or function may be a potential mechanism involved in the improvement of β-adrenergic responsiveness and of cardiac function in general.

The similarities between pressure overload–induced and CuD-induced cardiomyopathies were suggested previously given the similarity in the concentric nature of cardiac hypertrophy that develops in both models. It is reasonable to believe that the triggers leading to heart hypertrophy in both stimuli would be different; however, the signaling transduction pathways that mediate different etiologies could be shared extensively. This scenario was established in several studies, demonstrating that the same set of cardiac hypertrophic genes are upregulated by both pressure overload (24) and dietary CuD (4); these include β-myosin heavy chain, α-skeletal and α-smooth muscle actin, as well as atrial natriuretic peptide.

Multiple signaling pathways and cross-interactions between the pathways are involved in the regulation of cardiac hypertrophy (25). Therefore, both pressure overload and dietary CuD would activate the same cascade; once the cascade of signaling transduction that leads to hypertrophy is activated, cardiac hypertrophy would be a common end point. Accordingly, we assume that to prevent cardiac hypertrophy, an intervention targeting the etiology would be effective; however, to reverse the cardiac hypertrophy that has already developed, an etiology-targeted approach would not be effective. The result obtained from this study, however, demonstrates that heart hypertrophy induced by CuD can be reversed

by simply adding Cu back into the diet, an approach targeting the etiology. This is an unexpected result, and there is no doubt that CuR has to cause a shift from the myocardial remodeling to a reverse remodeling program because cardiac hypertrophy regressed, cardiac function improved, and the heart regained its responsiveness to adrenergic stimulation.

In the present study, our interest was in finding the effect on CuD cardiomyopathy of introducing Cu back into the diet, specifically focusing on the reversibility of damage to cardiac function and restoration of adrenergic responsiveness. This study is not the first reporting the possibility of amelioration of cardiomyopathy. Such phenomena were observed in patients with end-stage heart failure after mechanical circulatory support (26–30). There is substantial evidence showing the possibility of reverse remodeling of the heart after left ventricle assist device implantation (31). Such a process leads not only to improved geometry at the organ and cellular levels but also to improved left ventricular function as a result of a reduction in wall stress and improved mechanical performance (31). The observations obtained from the present study are quite similar to these clinical reports. However, there are fundamental differences in the mechanisms leading to the reverse remodeling. The left ventricle assist device is designed in such a way as to reduce left ventricle overload. In the CuR-induced reverse remodeling, Cu refeeding would not alter the loading conditions as such, but rather improve mineral status, which in turn would influence several components including those involved in contractility and relaxation. It appears that the reverse remodeling program might be shared in the processes of left ventricle assist device and CuR. Defining such a reverse remodeling program would be an important undertaking in the future.

Systemic changes in the CuR mice revealed that the improved myocardial morphology and function were associated with prevention of mortality and improved systemic physiology, as revealed by the regaining of lost body weight in the CuD mice and the overall improved health of the mice. The overall rescue from CuD-induced systemic changes such as mortality may not necessarily result exclusively from the reversed cardiac hypertrophy; the complete recovery of cardiac function would contribute significantly to this overall improvement.

In conclusion, the present study demonstrated that the transition from heart hypertrophy to end-stage failure induced by CuD was preventable upon reintroduction of Cu into the diet. Furthermore, CuR led to a reversal of CuD-induced heart hypertrophy along with improved myocardial histological and ultrastructural changes. These cardiac improvements were associated with improved systemic function and prevention of mortality induced by CuD. This study has opened a new avenue in the research of CuD-induced cardiomyopathy, namely, CuR-induced myocardial reverse remodeling.

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